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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Bisgaard-Frantzen et al.

Confirmation No: 7527

Serial No.: 09/902,188

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Examiner: To be assigned

For: Amylase Variants

PENDING CLAIMS

Commissioner for Patents Washington, DC 20231

- 68. A variant of a parent alpha-amylase enzyme, wherein said parent alpha-amylase has an amino acid sequence which has at least 80% homology to SEQ ID NO:3, and wherein said variant comprises deletions at positions equivalent to positions 180 and 181 in SEQ ID NO:3 (using SEQ ID NO:3 for numbering).
- 69. The variant of claim 68, wherein said parent alpha-amylase has an amino acid sequence which has at least 85% homology to SEQ ID NO:3.
- 70. The variant of claim 68, wherein said parent alpha-amylase has an amino acid sequence which has at least 90% homology to SEQ ID NO:3.
- 71. The variant of claim 68, wherein said parent alpha-amylase has an amino acid sequence which has at least 95% homology to SEQ ID NO:3.
- 72. The variant of claim 68, wherein said variant further comprises amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.
- 73. An isolated alpha-amylase enzyme comprising an amino acid sequence having an amino acid sequence which has at least 80% homology to SEQ ID NO:3, modified by having deletions at positions equivalent to positions 180 and 181 in SEQ ID NO:3.

- 74. The alpha-amylase enzyme of claim 73, wherein said alpha-amylase enzyme is further modified by having amino acid substitutions of a cysteine at positions equivalent to 349 and 428 in SEQ ID NO:3.
- 75. The alpha-amylase of claim 73, wherein said alpha-amylase has an amino acid sequence which has at least 85% homology to SEQ ID NO:3.
- 76. The alpha-amylase of claim 73, wherein said alpha-amylase has an amino acid sequence which has at least 90% homology to SEQ ID NO:3.
- 77. The alpha-amylase of claim 73, wherein said alpha-amylase has an amino acid sequence which has at least 95% homology to SEQ ID NO:3.
- 78. A process for producing an alpha-amylase enzyme, said process comprising:
- a) cultivating a host cell having a nucleic acid sequence encoding an alpha-amylase enzyme, said alpha-amylase enzyme comprising an amino acid sequence having at least 80% homology to SEQ ID NO:3 and wherein said alpha-amylase enzyme is modified by having deletions at positions equivalent to positions 180 and 181 in SEQ ID NO:3, wherein said cultivating is performed under conditions conducive to produce the alpha-amylase enzyme, and
 - b) recovering the alpha-amylase from the culture.
- 79. The process of claim 78, wherein said alpha-amylase enzyme is further modified by having amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.
- 80. A DNA construct having a DNA sequence encoding an alpha-amylase enzyme comprising an amino acid sequence of SEQ ID NO:3 and further comprising deletions at positions equivalent to positions 180 and 181 in SEQ ID NO:3.
- 81. The DNA construct of claim 80, wherein said alpha-amylase enzyme further comprises amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.
- 82. A recombinant expression vector carrying the DNA construct of claim 80.

- 83. A cell transformed with the DNA construct of claim 80.
- 84. A cell transformed with the recombination expression vector of claim 82.
- 85. A process of producing an alpha-amylase, said process comprising culturing the cell of claim 83 under conditions conducive for the production of the alpha-amylase and recovering the alpha-amylase from the culture.
- 86. A variant of a parent alpha-amylase enzyme, wherein said parent alpha-amylase has an amino acid sequence which has at least 80% homology to SEQ ID NO:3, and wherein said variant comprises deletions at positions equivalent to positions 179 and 181 in SEQ ID NO:3 (using SEQ ID NO:3 for numbering).
- 87. The variant of claim 86, wherein said parent alpha-amylase has an amino acid sequence which has at least 85% homology to SEQ ID NO:3.
- 88. The variant of claim 86, wherein said parent alpha-amylase has an amino acid sequence which has at least 90% homology to SEQ ID NO:3.
- 89. The variant of claim 86, wherein said parent alpha-amylase has an amino acid sequence which has at least 95% homology to SEQ ID NO:3.
- 90. The variant of claim 86, wherein said variant further comprises amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.
- 91. An isolated alpha-amylase enzyme comprising an amino acid sequence having an amino acid sequence which has at least 80% homology to SEQ ID NO:3, modified by having deletions at positions equivalent to positions 179 and 181 in SEQ ID NO:3.
- 92. The alpha-amylase enzyme of claim 91, wherein said alpha-amylase enzyme is further modified by having amino acid substitutions of a cysteine at positions equivalent to 349 and 428 in SEQ ID NO:3.

- 93. The alpha-amylase of claim 91, wherein said alpha-amylase has an amino acid sequence which has at least 85% homology to SEQ ID NO:3.
- 94. The alpha-amylase of claim 91, wherein said alpha-amylase has an amino acid sequence which has at least 90% homology to SEQ ID NO:3.
- 95. The alpha-amylase of claim 91, wherein said alpha-amylase has an amino acid sequence which has at least 95% homology to SEQ ID NO:3.
- 96. A process for producing an alpha-amylase enzyme, said process comprising:
- a) cultivating a host cell having a nucleic acid sequence encoding an alpha-amylase enzyme, said alpha-amylase enzyme comprising an amino acid sequence having at least 80% homology to SEQ ID NO:3 and wherein said alpha-amylase enzyme is modified by having deletions at positions equivalent to positions 179 and 181 in SEQ ID NO:3, wherein said cultivating is performed under conditions conducive to produce the alpha-amylase enzyme, and
 - b) recovering the alpha-amylase from the culture.
- 97. The process of claim 96, wherein said alpha-amylase enzyme is further modified by having amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.
- 98. A DNA construct having a DNA sequence encoding an alpha-amylase enzyme comprising an amino acid sequence of SEQ ID NO:3 and further comprising deletions at positions equivalent to positions 179 and 181 in SEQ ID NO:3.
- 99. The DNA construct of claim 98, wherein said alpha-amylase enzyme further comprises amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.
- 100. A recombinant expression vector carrying the DNA construct of claim 98.
- 101. A cell transformed with the DNA construct of claim 98.
- 102. A cell transformed with the recombination expression vector of claim 100.

- 103. A process of producing an alpha-amylase, said process comprising culturing the cell of claim 101 under conditions conducive for the production of the alpha-amylase and recovering the alpha-amylase from the culture.
- 104. A variant of a parent alpha-amylase enzyme, wherein said parent alpha-amylase has an amino acid sequence which has at least 80% homology to SEQ ID NO:3, and wherein said variant comprises deletions at positions equivalent to positions 179 and 182 in SEQ ID NO:3 (using SEQ ID NO:3 for numbering).
- 105. The variant of claim 104, wherein said parent alpha-amylase has an amino acid sequence which has at least 85% homology to SEQ ID NO:3.
- 106. The variant of claim 104, wherein said parent alpha-amylase has an amino acid sequence which has at least 90% homology to SEQ ID NO:3.
- 107. The variant of claim 104, wherein said parent alpha-amylase has an amino acid sequence which has at least 95% homology to SEQ ID NO:3.
- 108. The variant of claim 104, wherein said variant further comprises amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.
- 109. An isolated alpha-amylase enzyme comprising an amino acid sequence having an amino acid sequence which has at least 80% homology to SEQ ID NO:3, modified by having deletions at positions equivalent to positions 179 and 182 in SEQ ID NO:3.
- 110. The alpha-amylase enzyme of claim 109, wherein said alpha-amylase enzyme is further modified by having amino acid substitutions of a cysteine at positions equivalent to 349 and 428 in SEQ ID NO:3.
- 111. The alpha-amylase of claim 109, wherein said alpha-amylase has an amino acid sequence which has at least 85% homology to SEQ ID NO:3.

- 112. The alpha-amylase of claim 109, wherein said alpha-amylase has an amino acid sequence which has at least 90% homology to SEQ ID NO:3.
- 113. The alpha-amylase of claim 109, wherein said alpha-amylase has an amino acid sequence which has at least 95% homology to SEQ ID NO:3.
- 114. A process for producing an alpha-amylase enzyme, said process comprising:
- a) cultivating a host cell having a nucleic acid sequence encoding an alpha-amylase enzyme, said alpha-amylase enzyme comprising an amino acid sequence having at least 80% homology to SEQ ID NO:3 and wherein said alpha-amylase enzyme is modified by having deletions at positions equivalent to positions 179 and 182 in SEQ ID NO:3, wherein said cultivating is performed under conditions conducive to produce the alpha-amylase enzyme, and
 - b) recovering the alpha-amylase from the culture.
- 115. The process of claim 114, wherein said alpha-amylase enzyme is further modified by having amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.
- 116. A DNA construct having a DNA sequence encoding an alpha-amylase enzyme comprising an amino acid sequence of SEQ ID NO:3 and further comprising deletions at positions equivalent to positions 179 and 182 in SEQ ID NO:3.
- 117. The DNA construct of claim 116, wherein said alpha-amylase enzyme further comprises amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.
- 118. A recombinant expression vector carrying the DNA construct of claim 116.
- 119. A cell transformed with the DNA construct of claim 116.
- 120. A cell transformed with the recombination expression vector of claim 118.

- 121. A process of producing an alpha-amylase, said process comprising culturing the cell of claim 119 under conditions conducive for the production of the alpha-amylase and recovering the alpha-amylase from the culture.
- 122. A variant of a parent alpha-amylase enzyme, wherein said parent alpha-amylase has an amino acid sequence which has at least 80% homology to SEQ ID NO:3, and wherein said variant comprises deletions at positions equivalent to positions 180 and 182 in SEQ ID NO:3 (using SEQ ID NO:3 for numbering).
- 123. The variant of claim 122, wherein said parent alpha-amylase has an amino acid sequence which has at least 85% homology to SEQ ID NO:3.
- 124. The variant of claim 122, wherein said parent alpha-amylase has an amino acid sequence which has at least 90% homology to SEQ ID NO:3.
- 125. The variant of claim 122, wherein said parent alpha-amylase has an amino acid sequence which has at least 95% homology to SEQ ID NO:3.
- 126. The variant of claim 122, wherein said variant further comprises amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.
- 127. An isolated alpha-amylase enzyme comprising an amino acid sequence having an amino acid sequence which has at least 80% homology to SEQ ID NO:3, modified by having deletions at positions equivalent to positions 180 and 182 in SEQ ID NO:3.
- 128. The alpha-amylase enzyme of claim 127, wherein said alpha-amylase enzyme is further modified by having amino acid substitutions of a cysteine at positions equivalent to 349 and 428 in SEQ ID NO:3.
- 129. The alpha-amylase of claim 127, wherein said alpha-amylase has an amino acid sequence which has at least 85% homology to SEQ ID NO:3.
- 130. The alpha-amylase of claim 127, wherein said alpha-amylase has an amino acid sequence which has at least 90% homology to SEQ ID NO:3.

- 131. The alpha-amylase of claim 127, wherein said alpha-amylase has an amino acid sequence which has at least 95% homology to SEQ ID NO:3.
- 132. A process for producing an alpha-amylase enzyme, said process comprising:
- a) cultivating a host cell having a nucleic acid sequence encoding an alpha-amylase enzyme, said alpha-amylase enzyme comprising an amino acid sequence having at least 80% homology to SEQ ID NO:3 and wherein said alpha-amylase enzyme is modified by having deletions at positions equivalent to positions 180 and 182 in SEQ ID NO:3, wherein said cultivating is performed under conditions conducive to produce the alpha-amylase enzyme, and
 - b) recovering the alpha-amylase from the culture.
- 133. The process of claim 132, wherein said alpha-amylase enzyme is further modified by having amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.
- 134. A DNA construct having a DNA sequence encoding an alpha-amylase enzyme comprising an amino acid sequence of SEQ ID NO:3 and further comprising deletions at positions equivalent to positions 180 and 182 in SEQ ID NO:3.
- 135. The DNA construct of claim 134, wherein said alpha-amylase enzyme further comprises amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.
- 136. A recombinant expression vector carrying the DNA construct of claim 134.
- 137. A cell transformed with the DNA construct of claim 134.
- 138. A cell transformed with the recombination expression vector of claim 136.
- 139. A process of producing an alpha-amylase, said process comprising culturing the cell of claim 137 under conditions conducive for the production of the alpha-amylase and recovering the alpha-amylase from the culture.